

Effect of defatted green tea extract, polyphenol fraction and other compounds present in green tea extract on the photostability of deltamethrin applied to cotton discs.

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ABSTRACT

GC-MS analysis showed that the major constituents of green tea extract were heptadecene-(8)-carboxylic acid, 4-o-methyl gallic acid, procatechuic acid, and small amount of caffeine. Defatted methanolic green tea extract and its ethyl acetate fractions (polyphenol extract) divided to groups [green tea extract and major constituents] were examined for their effect in an increasing the photostability of deltamethrin insecticide under simulating sun radiation condition. In the absence of the UV absorber, only 5.4% of the initial amount of deltamethrin applied on cotton disc screen was recovered after the six hours of irradiation under simulating sunlight (equivalent to 96 hour of natural sunlight at mid day). Whereas, $96.800 \pm 1.2\%$ was recovered from the control sample kept in the dark at 35°C . However, A_5 [(+) - catechin] was the most effective compound acting as UVabsorber among the the UV absorber tested. It provided more protection to deltamethrin against photolysis process. 38.2% of the initial amount applied to the target screen that exposed to UV radiation for 6 hours was recovered and 61.8% deltamethrin was lost by photolysis in the presence of (+) – catechin. The other compounds could arranged in decreasing order, according to the degree of protection performance, were tannic acid, green tea Polyphenols, defatted green tea extract and caffeine.

Keywords: Green tea, polyphenol, photostability, deltamethrin.

INTRODUCTION

Pesticides are used extensively in agriculture, as their application is still the most effective means for controlling pest populations. However, overexposure to pesticides worldwide has been accompanied with problems related to toxic and genotoxic effects at sublethal concentrations to beneficial arthropods and non-target organisms, and health risks for humans (Dearfield *et al.*, 1999 & Bolognesi, 2003). Moreover, extensive and long-term use of insecticides may lead to insecticide resistance problems (Toshio and Scott 2003; Kakaniet *et al.*, 2010). Deltamethrin; (S)- α -cyano-3-phenoxybenzyl-cis

(1*R*,3*R*) -2,2-dimethyl-3 (2,dibromovinyl)-2,2-dimethyl cyclopropane carboxylate] is a synthetic pyrethroid insecticide was well known late 1970s and early 1980 as NRDC161 and decamethrin. The insecticide is active against a wide range of insects which attack crops, animals, and mankind. The greater stability of this compound than other earlier alternative pyrethroids has made it adequate for uses requiring longer residual activity (Bengston *et al.*, 1983). Currently deltamethrin is recommended by the World Health Organization Pesticide Evaluation Schemes (WHOPES) to overcome insect transmitted diseases such as malaria and

yellow fever. Insecticide-treated nets impregnated with deltamethrin have become one of the most promising interventions to prevent malaria in highly endemic areas (Sharma *et al.*, 2005). This insecticide reported to show the highest selectivity towards fly such as tsetse in the form of impregnated insecticide target, Torr (1985). The most important factor affecting net effectiveness under field conditions is the residual concentration remaining on the net impregnated with deltamethrin that subjected to sunlight and water wash. Many investigators suggested that the use of photostabilizer in the formulations to reducing the photodegradation rate of deltamethrin (Hussain *et al.*, 1990; Hussain and priskei, 1994). However, applying insecticides are lower in active ingredient content than declared could result in monetary loss and application of sub-lethal dose of insecticide, leading to ineffective control and promotion of resistance development (Baraud *et al.*, 2003).

The present work outlines the result of studies conducted to determine the effect of selected natural UV absorber extracted from the green tea on the stability of deltamethrin applied to cotton fabric screen and subjected to the effect of simulated UV-sunlight.

MATERIALS AND METHODS

Extraction, identification and confirmation of certain UV absorbers present in green tea

The extraction technique was carried out according to method described by Cannell (1998). Dried green tea leaf 50 g was extracted by 250 ml solvent mixture consisting of methanol: water at ratio 8:2 (V/V) using soxhlet apparatus for eight

hours. The combined aqueous methanolic crude extract was filtered and then subjected to activated charcoal to remove the chlorophyll and sticky materials. The extract was concentrated to small volume 100 ml under reduced pressure (40°C) by rotary evaporator, followed by partitioning three times using 100 ml petroleum ether (40-60°C) for each time to remove any fatty materials, alkanes and waxesto obtain the MeOH aqueous extract termed defatted methanolic crude extract. The defatted methanolic crude extract was extracted once again with 100 ml chloroform to obtain a group of compounds such as polyphenols, alkaloids and saponins.

Separation of phenolic compounds was carried out according to the method described by Hussein *et al.* (1999) with slightly modification. Defatted methanolic crude extract (20 ml) was used to extract polyphenolic by using acid hydrolysis (2N HCl; 10mL) for 30 minutes in water bath. The extract was left to cool at room temperature and the filtrate was partitioned three times in a separatory funnel using 20 ml ethyl acetate for each time. The organic layer was collected and washed, dried under nitrogen stream and the remaining crystal after evaporation process termed polyphenol. The total amount of polyphenols extracted was 120 mg. Twenty milligram of polyphenols was redissolved in 5 ml ethyl acetate to give a total concentration of 4000 ppm to identify the major chemical constituent by using gas liquid chromatography equipped with mass spectrometry (GC-MS).

GC-MS analysis of green tea polyphenol extract

The chemical constituents of polyphenol extracts were made using Hewllet Paekard

(HP5890) gas liquid chromatography equipped with flame ionization detector (GC-FID) coupled with Hewlett Paekard (HP5890B) series mass spectrometer (MS) under the following conditions; the sample injected with split ratio 1:10 in sample injector set at 240 ° C, and flame ionization detector (FID) was held at 260 ° C and helium was used as carrier gas with flow rate 1ml/min. Sample was separated on capillary column [30m, and 0.25mm internal diameter coated with HP-5 (5% diphenyl 95% dimethyl polysiloxane). GC-Column temperature was programmed from 50 ° C to 180 ° C at heating rate of 25° per minute, the maximum final temperature was held for a residence time of 1 min. then the temperature raised to 240° C by using heating rate of 20° C/minute, the maximum final temperature was held for a residence time of 1 min. The temperature of ion source in the mass spectrometer was held at 200° C. A Hewlett Paekard mass spectrometer was operated in electron impact ionization (EI) at 70 electron volts [ev]. The mass spectrometer was scanned from m/z 50 to 500 at rate of two scans per second, and peak area was calculated by integrator worked automatically. Neither internal nor external chemical standards were used in this chromatographic analysis. Interpretation of the resultant mass spectra were made by using a computerized library searching program, as well as by studying the fragmentation of such compound resulted from mass spectrometry analysis (Table 1).

Screening of UV absorbers mixed with deltamethrin on cotton discs under simulating UV sun radiation

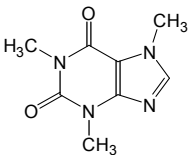
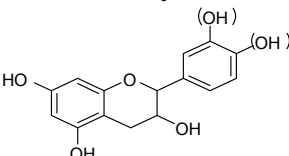
Irradiation of deltamethrin impregnated cotton target with simulating UV sun radiation was carried out according to method described by Hussain *et al.*, (1990) with slightly modification. Circle cotton targets 2 cm diameter were bunched out from white cotton (100%) fabrics were treated with aliquot of insecticide (50 μ L dioxan containing 100 μ g deltamethrin) mixed with 100 μ g of the tested UV absorber (Table 2). Each treatment replicated three times. After the solvent had been evaporated [approximately one hours], the cotton target were placed on aluminium spread sheet placed on stainless steel platform which provided with cooling system to controlling and to maintaining a constant temperature of 35 ± 1 °C. The platform was positioned under a UV sun radiation lamp (300 W, H. I. D. Nippon, mercury lamp) to eradiate the target samples. The lamp emitted radiation simulating the spectral distribution of natural sunlight. The lamp is designed to provide radiation intensity equivalent to med-day natural sunlight ($1\text{kW m}^{-2} = 1.42\text{ cal cm}^{-2}\text{ min}^{-1}$) at the irradiated surface when the lamp is adjusted 50 cm above the surface. The lamp was positioned 12.5 cm above the samples and provided radiation intensity equivalent to approximately 16 times that mid-day natural sun light. The fabric cotton discs treated with deltamethrin and UV absorber were irradiated for a period 0.0, 45, 90, 180, and 360 min. to give simulation period to 0.0, 12, 24, 48 and 96 hrs of the mid-day natural sunlight. Fifteen cotton discs were irradiated as mentioned above each containing 100 μ g deltamethrin alone to calculate the half life (t_{50}) of insecticide as control samples. Three cotton discs, each containing 100 μ g deltamethrin alone were kept in the dark at 35 ± 1 °C on as control sample to examine the effect of the temperature on the loss of insecticide.

Table 1. GC-MS retention time and chemical name of major constituent in ethyl acetate fraction of green tea extract

Chemical name	RT ^a	C % ^b	MW ^c
Procatechuic acid [3,4-dihydroxy benzoic acid)	2.21	10.72	154
Caffeine	4.04	5.53	194
4-o-methyl gallic acid	4.40	7.69	184
Hexadecanoic acid	4.98	41.02	256
Heptadecene-(8)-carbonic acid	5.30	23.48	282
Stearic acid	5.54	11.56	284

a: peak retention time, b: concentration of the component based on hole peaks area and c: molecular weight of the components.

Table 2. Code number and chemical structure of major constituent of polyphenol green tea extract that was tested as UV absorber with deltamethrin at ratio [1:1] weight by weight

Code No.	Chemical name	Source	Chemical structure
A ₁	Defatted green tea extract	extracted	--
A ₂	Green tea Poly phenols	extracted	--
A ₃	Tannic Acid	BDH	--
A ₄	3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6,-dione Caffeine	BDH	
A ₅	(+)-Catechin	Serva	

Deltamethrin extraction and sample preparation

Deltamethrin remained was extracted twice with 2 ml acetone for each time by shaking for 4 minute each time. Acetone extracts were combined, and then two drops (15-25µL) of ethylene glycol as trap solvent was added to each vial. To avoid glass adsorption of deltamethrin; the vials were treated with 5% (w/v) poly ethylene glycol (PEG; molecular weight 20000), according to the method described by Helmuth *et al.*, (1983). The remaining deltamethrin residues were

redissolved by addition of one ml methanol to each vial after the acetone had been stripped by stream of N₂ then vials were kept at 0.0 °C until the monitoring of deltamethrin concentration by enzymatic-linked immunosorbent assay (ELISA) technique that was developed and optimized for deltamethrin analysis by Soltan *et al.*, (2009).

Calculation of deltamethrin and its mixture with UV absorber half life (*t*₅₀) values

The data collected for either the deltamethrin alone or its mixture with the UV-absorbers at time intervals which irradiated to simulating exposure to mid-day sunlight on cotton discs were plotted on GraphPad Prism program version 4.00. The dissipation rate constant [K] of deltamethrin as well as the half life values were calculated from the first order reaction on of the first order dissipation kinetics.

Statistical analysis

The statistical analysis of variance between treatment after 6 hrs of irradiation with simulating UV sun radiation were recorded using analysis of variance [ANOVA analysis] followed by Tukey's Multiple Comparison Test to calculate the significantly deference between treatment by using GraphPad Prism program version 4 (2002).

RESULTS AND DISCUSSION

Effect of selected UV absorber compounds on the photolysis of deltamethrin

Data shown in Table (3) and Figure (1) indicated that in the absence of UV absorbers, deltamethrin degraded rapidly on the target screen when exposed to simulating sun radiation. The dissipation rate constant calculated was 0.01913 min^{-1} and the correlation coefficient was 0.9668 which indicated that the photodegradation rate follows first order kinetics. On the other hand, deltamethrin degradation was minimal during storage at 35°C in the dark and $96.800 \pm 1.2 \%$ of the deltamethrin applied to the cotton target after six hours storage was recovered, Most of this loss might be due to the formation volatile photoproducts and these photoproducts were not

identified (Hussain *et al.*, 1990). In contrary, 5.4% of the deltamethrin was recovered after the six hours of irradiation under simulating sunlight (equivalent to 96 hour of natural sunlight at mid day).

The effect of selected natural UV absorbers on the photolysis rate of deltamethrin

Identification and confirmation by using GC-MS showed that chemicals compounds in ethyl acetate fraction extracted after acid hydrolysis from green tea leaves *Camellia sinensis* had six components (Table 1). Hexadecanoic acid with molecular weight 256 was the major constituent in this fraction with the yield (41.02%). It was also found that ethyl acetate fraction of *C. sinensis* contained theheptadecene-(8)-carbonic acid (MW = 282) in high value 23.48. In addition, stearic acid, procatechuic acid, methyl ester of gallic acid and little amount of caffeine were presented in the ethyl acetate fraction. This study was carried out to find out the ability of major constituent of green tea leaves and ethyl acetate fraction extract from green tea crude extract to protect the deltamethrin insecticide against photodegradation. Data shown in Table (3) indicated that in the absence of UV absorbers deltamethrin degraded rapidly on the target screen when exposed to simulating sun radiation. The dissipation rate constant calculated was 0.01913 min^{-1} and the correlation coefficient was 0.9668 which indicated that the photodegradation rate follows first order kinetics. On the other hand degradation during storage at 35°C in the dark was minimal and $96.800 \pm 1.2 \%$ of the deltamethrin applied to the cotton target after six hours storage was recovered.

This loss might be due to the formation volatile photoproducts and these photoproducts were not identified (Hussain *et al.*, 1990). Also, data shown Figure (1) indicated that there are

variations among the UV absorbers tested on the stability of deltamethrin applied to cotton fabric screens and subjected to simulated sunlight for six hours.

Table 3. Effect of simulating sun radiation on the photolstability of deltamethrin

Treatment	exposure time (min)	% of Deltamethrin Recovery*± SD	Half life (min)
DM	0.0	97.00 ±3.60	36.1
	45	33.80 ±2.63	
	90	21.70 ±2.13	
	180	11.20 ±1.73	
	360	5.40 ±0.68	
DM ^c	360	96.80 ±1.2	

DM: Deltamethrin alone, DM^c: Deltamethrin alone kept in the dark at 35 ± 1° C,*: value means of three replicates with standard deviation recovered from cotton disc have initial concentration [31.83 µg cm⁻¹] as percent %.

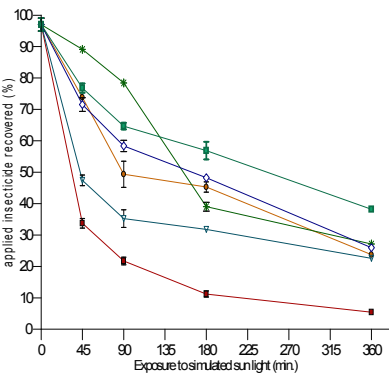


Figure 1. Recovery of unchanged deltamethrin from cotton fabric treated with (■) deltamethrin alone, (●) deltamethrin + UV absorber A₁ [1:1], (✱) deltamethrin + UV absorber A₂ [1:1], (◇) deltamethrin + UV absorber A₃ [1:1], (■) deltamethrin + UV absorber A₅ [1:1] and (▼) deltamethrin + UV absorber A₄ [1:1].

According to statistical analysis tabulated in Table (4) all the UV absorbers were highly significant ($p<0.0001$) on the protection of deltamethrin against photolysis comparable to deltamethrin applied alone without UV absorber. However, A₅ [(+)-Catechin] was the most effective compound on protection of deltamethrin against photolysis and 38.2% of the deltamethrin applied to the target screen exposed to UV radiation for 6 hours was recovered. Thus 61.8% deltamethrin was lost by photolysis in the presence of A₅, the other compounds in decreasing order of performance were A₃, A₂, A₁ and A₄. The half-life values calculated and presented in Table (4) revealed that the mixture of deltamethrin with A₁ [Defatted green tea extract] at the rate of 1:1 w/w exhibited positive protection effect and the half life value of deltamethrin increased by 4.28 times than without UV absorbers.

Table 4. Effect of green tea extract, polyphenol and major constituent of polyphenol green tea extract on the photolysis of deltamethrin

Treatment	exposure time (min)	% of Deltamethrin Recovery* $\pm$ SD	Half life ^π (min)
DM	0.0	97.00 $\pm$ 3.60	36.1
	45	33.80 $\pm$ 2.63	
	90	21.70 $\pm$ 2.13	
	180	11.20 $\pm$ 1.73	
	360	5.40 $\pm$ 0.68 ^a	
DM+A ₁	0.0	97.00 $\pm$ 3.60	154.07
	45	73.80 $\pm$ 0.72	
	90	49.30 $\pm$ 7.19	
	180	45.28 $\pm$ 2.88	
	360	23.76 $\pm$ 0.73 ^b	
DM+A ₂	0.0	97.00 $\pm$ 3.60	172.07
	45	89.13 $\pm$ 0.84	
	90	78.43 $\pm$ 0.07	
	180	39.03 $\pm$ 2.45	
	360	27.12 $\pm$ 0.58 ^c	
DM+A ₃	0.0	97.00 $\pm$ 3.61	178.3
	45	71.53 $\pm$ 3.76	
	90	58.38 $\pm$ 3.11	
	180	48.24 $\pm$ 1.00	
	360	25.94 $\pm$ 1.81 ^{b,c}	
DM+A ₄	0.0	97.00 $\pm$ 3.61	92.28
	45	47.40 $\pm$ 2.96	
	90	35.24 $\pm$ 4.86	
	180	31.77 $\pm$ 0.23	
	360	22.59 $\pm$ 1.52 ^b	
DM+A ₅	0.0	97.00 $\pm$ 3.6	256.2
	45	76.79 $\pm$ 2.5	
	90	64.67 $\pm$ 1.87	
	180	56.87 $\pm$ 3.98	
	360	38.20 $\pm$ 0.0 ^d	

DM: Deltamethrin, DM+A₁ (Defatted green tea extract), A₂ (Green tea Poly phenols), A₃ (Tannic Acid), A₄ (Caffeine), A₅ ((+)-Catechin); Deltamethrin mixtures with tested UV absorber at ratio [1:1 W/W], *: Mean of three replicates with initial concentration on cotton disc [31.83 μ g cm⁻¹] as percent %, π: One hour of irradiation with simulated sun radiation at distance 12.5 cm equivalent to 16h of natural sun radiation at mid day, and means with same letter did not show any significantly different with p<0.05.

Also A₂ [polyphenol extract] and A₃ showed significant effect on the protection of deltamethrin against deltamethrin alone, and the half life were ranged from 172.07 min. to 178.3 min. This result expected since the result of GC-MS showed that procatechuic acid, methyl ester of gallic acid and little amount of caffeine were presented in the ethyl acetate fraction which might be have effective role in protection of deltamethrin against simulating sun radiation. On the other hand no significant difference found

on the protection effect of A₂ against A₃ on deltamethrin photodegradation rate. A₄ did not show significant variance on the protection of deltamethrin against photolysis comparable with A₁ and A₂ although it was the worst one in this concern. A₅ was a highly significant one among the other UV absorbers and 38.2% of the original amount of deltamethrin applied to the target screen and exposed to UV radiation for six hours was recovered with half life was 256.2 min. This means that the persistence of deltamethrin

was increased 7 times than deltamethrin applied without UV absorbers. The photodegradation rate of deltamethrin was decreased when A₅ was incorporated with. This could be attributed to the quenching of UV absorbed by deltamethrin. This was confirmed from the UV absorption spectrum of deltamethrin that exhibits four peaks at 199, 204, 246 and 279 nm with higher molar absorption coefficient at 246 nm. On the other hand, the UV spectrum of the UV absorber exhibits three peaks at 218, 238 and 282 with higher molar absorption coefficient at 238 nm.

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تأثير مستخلص الشاي الأخضر منزوع الدهن، مركبات البوليفينول المفصولة من المستخلص ومركبات أخرى موجودة في مستخلص الشاي الأخضر على معدل التحطم الضوئي لمبيد الدلتاميثرين

الملخص العربي

الاستخلاص والتعرف على المواد الممتصة للأشعة فوق بنفسجية من المصادر الطبيعية مثل مستخلص عديد الفينول من الشاي الأخضر و التعرف عليها باستخدام التحليل الكروماتوجرافي الغازي المزود بمطياف الكتلة نُفذت في هذه الدراسة وذلك للتحقق من المكونات الكيميائية الرئيسية في هذا المصدر الطبيعي. ومن خلال التحليل الكروماتوجرافي الغازي تم التعرف على العديد من المواد الرئيسية في مستخلص عديد الفينول للشاي الأخضر هي heptadecene-(8)-carbonic acid, 4-o-methyl gallic acid and procatechuic acid وكمية صغيرة من الـ caffeine. تم اختبار المستخلص الميثانولي للشاي الأخضر المنزوع الدهن ومستخلص خلاص الايثيل للشاي الأخضر (عديد الفينول) ثم تم تقسيمهم الى مجموعات (المستخلص الميثانولي للشاي الأخضر المنزوع الدهن ومستخلص عديد الفينول بالإضافة إلى المواد الرئيسية الموجودة فيه. تم دراسة تأثيرهم في زيادة الثبات الضوئي لمبيد الدلتاميثرين على اقراص قطنية بيضاء دائرية تحت ظروف تحاكي الإشعاع الشمسي. أظهرت النتائج المتحصل عليها أنه في غياب المواد الممتصة للأشعة فوق بنفسجية فإن تركيز المتبقي من مبيد الدلتاميثرين ينخفض بسرعة بزيادة فترات التعرض وجد أن النسبة المئوية المتبقية 5.4% من الكمية الأولية المطبقة بعد 6 ساعات من التعرض الى لمبة الأشعة فوق البنفسجية (تكافئ 96 ساعة من اشعة الشمس الطبيعية) إلا أنه عند إضافة المواد الممتصة للأشعة فوق بنفسجية اظهرت هذه المركبات نشاطا ملحوظا في حماية الدلتاميثرين من التحطم الضوئي بدرجات متفاوتة وكان اكثر المركبات قدرة على حماية الدلتاميثرين من التحطم الضوئي هو مركب Catechin (+) وكانت النسبة المئوية من متبقى المبيد تصل الى 38.2% والنسبة المئوية للفقء تصل الى 61.8% نتيجة التحطم الضوئي. بينما كانت الكمية المسترجعة من عينات الكونترول (الموجودة في الظلام) تصل الى 96.8% من الكمية المطبقة. ويمكن ترتيب باقي المركبات طبقا لقدرتها على حماية المبيد من التحطم الضوئي بترتيب تنازلي على النحو التالي: حامض التنيك ومركبات عديد الفينول المستخلصة من الشاي الأخضر ثم أخيراً الكافيين.